

[ CASE REPORT ]

## A Late-onset and Relatively Rapidly Progressive Case of Pure Spinal Form Cerebrotendinous Xanthomatosis with a Novel Mutation in the *CYP27A1* Gene

Ken Takasone<sup>1</sup>, Teruya Morizumi<sup>1</sup>, Katsuya Nakamura<sup>1,2</sup>, Yusuke Mochizuki<sup>1</sup>,  
Tsuneaki Yoshinaga<sup>1</sup>, Shingo Koyama<sup>3</sup> and Yoshiki Sekijima<sup>1</sup>

### Abstract:

A 61-year-old Japanese man with the pure spinal form of cerebrotendinous xanthomatosis developed dysesthesia of the lower limbs and gait disturbance at 57 years of age. At 61 years old, he was unable to walk without support. A neurological examination showed spasticity and sensory disturbance in the lower limbs. Spinal MRI showed long hyperintense lesions involving the lateral and posterior funiculus in the cervical and thoracic cord on T2-weighted images. His serum cholestanol level was markedly elevated. A *CYP27A1* gene analysis identified two missense variants, p.R474W, and a novel p.R262C variant. Combination therapy with chenodeoxycholic acid and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase decreased his serum cholestanol level.

**Key words:** cerebrotendinous xanthomatosis, *CYP27A1* gene, cholestanol, myelopathy, pure spinal form, late onset

(Intern Med 59: 2587-2591, 2020)

(DOI: 10.2169/internalmedicine.5037-20)

### Introduction

Cerebrotendinous xanthomatosis (CTX, OMIM 213700) is an autosomal recessive inherited lipid storage disease caused by a variant of the *CYP27A1* gene (1-3). *CYP27A1* encodes sterol 27-hydroxylase, which metabolizes cholesterol into bile acid. Therefore, its deficiency results in a decreased synthesis of bile acids, predominantly chenodeoxycholic acid (CDCA), and an increased production of cholesterol metabolites, including cholestanol (2). The absence of a negative feedback mechanism by CDCA on cholesterol 7 $\alpha$ -hydroxylase accelerates these metabolic abnormalities (3). Cholesterol metabolites subsequently accumulate in many tissues, particularly in the brain, tendons, eye lenses, vessels, and bones. CTX is a multisystem disorder characterized by tendon xanthoma, neonatal cholestatic jaundice, chronic refractory diarrhea, juvenile cataract, coronary artery disease,

and juvenile osteoporosis. In addition, neurological manifestations, including intellectual disability/cognitive impairment, ataxia, epilepsy, spastic paraplegia, parkinsonism, and peripheral neuropathy are seen in patients with CTX. The clinical phenotypes of CTX are categorized into three forms: classical, spinal, and non-neurological forms. Spinal form patients chiefly display chronic myelopathy (4). Recently, pure spinal form CTX patients have been reported to only show a slowly progressive myelopathy (4-8), and such patients are typically misdiagnosed with hereditary spastic paraplegia. We herein described a late-onset, relatively rapidly progressive pure spinal form CTX patient with a novel variant in the *CYP27A1* gene.

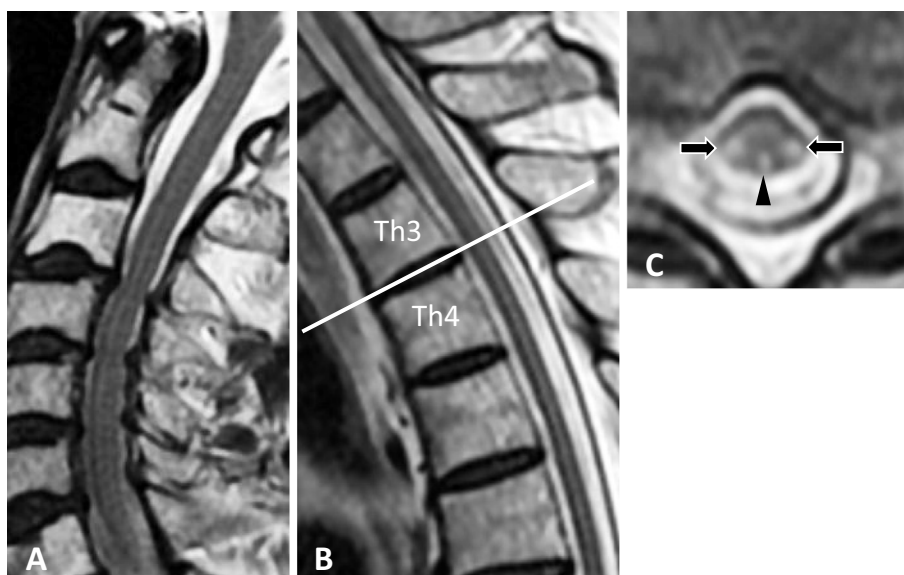
### Case Report

The patient was a 61-year-old Japanese man with a medical history of diffuse large B-cell lymphoma. He drank sake

<sup>1</sup>Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine, Japan, <sup>2</sup>Center for Medical Genetics, Shinshu University Hospital, Japan and <sup>3</sup>Department of Neurology, Hematology, Metabolism, Endocrinology, and Diabetology, Yamagata University Faculty of Medicine, Japan

Received: April 6, 2020; Accepted: May 6, 2020; Advance Publication by J-STAGE: June 23, 2020

Correspondence to Dr. Katsuya Nakamura, katsuya@shinshu-u.ac.jp



**Figure 1.** Spinal cord magnetic resonance imaging (MRI) of the patient (T2-weighted image). (A) Cervical cord, sagittal view (B) Thoracic cord, sagittal view. (C) Thoracic cord, axial view. Long hyperintense lesions involving the lateral corticospinal tracts (arrow) and dorsal corticospinal tracts (arrowhead) were observed.

frequently, equivalent to an absolute alcohol of 108 g/day every day. He was a former smoker, smoking 1 pack of cigarettes a day, for 40 years. He was the second child of non-consanguineous, unaffected parents, and had an unaffected sibling. He was born at term after a normal pregnancy, and showed normal development. Neither jaundice nor chronic diarrhea were seen. He had been employed as a manufacturing worker until he was 57-year-old, when he developed dysesthesia of the lower legs and gait disturbance. At 61 years of age, he was unable to walk without assistance, and was admitted to our hospital.

On admission, the patient's general examination was unremarkable, except for cataracts appropriate for his age. Achilles tendon xanthomas were absent. A neurological examination revealed that he was alert and well oriented. No abnormal findings in the cranial nerves were observed. There was no muscle weakness in his upper or lower limbs; however, he did experience decreased light touch, pain, and vibration sensation in the lower limbs. Romberg's sign was positive. Increased deep tendon reflexes of the limbs, ankle clonus, and positivity for Babinski and Chaddock signs were observed on both sides. His gait was spastic and ataxic, and was unable to walk without support (expanded disability status scale, EDSS 7). His Mini-Mental State Examination (MMSE) score was 30. Routine blood tests showed increased serum aspartate aminotransferase (AST) levels of 51 IU/L, and  $\gamma$ -glutamyl transpeptidase ( $\gamma$ GTP) levels of 344 IU/L, likely due to alcoholic liver injury. Serum folic acid, vitamin B<sub>12</sub>, copper, ceruloplasmin, and plasma very long chain fatty acids were all within the normal ranges. Serum anti-aquaporin-4 (AQP4) antibody was negative. The findings of cerebrospinal fluid, chest roentgenography, and electrocardiography were unremarkable. However, a marked ele-

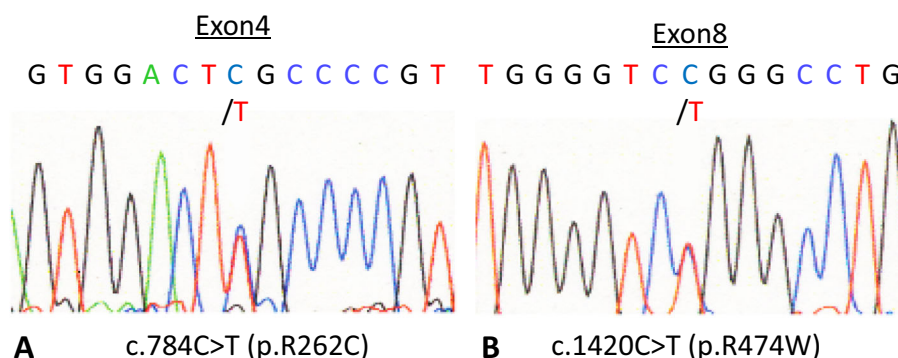
vation of the serum cholestanol levels was observed (14.1  $\mu$ g/mL; normal, 1.91 - 3.51  $\mu$ g/mL). Xanthoma was detected neither on X-ray nor gallium-67 (67Ga) scintigraphy in the Achilles tendons. Bone mineral density at the lumbar spine was intact, as assessed by dual-energy X-ray absorptiometry. Nerve conduction studies were normal, whereas the motor evoked potential (MEP) in the lower limbs revealed a prolonged central motor conduction time. The somatosensory evoked potential (SEP) in the lower limbs also showed a prolonged central sensory conduction time. Brain magnetic resonance imaging (MRI) was unremarkable. Spinal cord MRI showed long hyperintense lesions involving the lateral and posterior funiculus in the cervical and thoracic cord, extending from C2 to Th11 level on T2-weighted images (Fig. 1).

As clinical manifestations, increased serum cholestanol levels and spinal cord MRI findings were suggestive of pure spinal form CTX. Genetic testing for the *CYP27A1* gene was performed after informed consent was obtained. DNA was extracted from the peripheral leukocytes of the patient according to the standard protocol. All nine exons of the *CYP27A1* gene were amplified by polymerase chain reaction (PCR). A direct sequence analysis of the PCR-amplified DNA from the patient identified two heterozygous missense variants, c.784C>T (p.R262C) (Fig. 2A), and c.1420C>T (p.R474W) (Fig. 2B). The c.1420C>T (p.R474W) is the fourth most common variant in Japanese CTX patients (4, 9-11), whereas the c.784C>T (p.R262C) variant has not been described in disease-causing mutation databases, such as the Human Gene Mutation Database (HGMD) Professional (<http://www.hgmd.org/>) and ClinVar (<http://www.ncbi.nlm.nih.gov/clinvar/>). The possible impact of the novel p.R262C variant on the structure and function of sterol

**Table. Allele Frequency and Results of In-silico Analysis of CYP27A1 Variants Identified in This Study.**

Base change	AA change	dbSNP	1000G Freq	ExAC Freq	SIFT	Polyphen2	MutationTester	CADD
c.784C>T	p.R262C	rs7783713	0	0.00006589	D	D	D	33
c.1420C>T	p.R474W	rs1219080	0	0.00001663	D	D	A	35

AA Change: amino acid change, dbSNP#: The Single Nucleotide Polymorphism Database reference number, 1000G: the 1000 Genomes Project (<http://www.internationalgenome.org>), Freq: allele frequency, ExAC: the Exome Aggregation Consortium (<http://exac.broadinstitute.org/>), D: deleterious, A: disease-causing-automatic



**Figure 2.** Sanger sequences of *CYP27A1*. A heterozygous previously reported missense variant (c.784C>T, p.R262C) and a novel missense variant (c.1420C>T, p.R474W) were found in this patient.

species	R262	R474
Human	SLYATFLPKWTRPVLPFWKRYLDG	PFGSVPFYGVRAACLGRRIAELEM
Chimpanzee	SLYATFLPKWTRPVLPFWKRYLD	PFGSVPFYGVRAACLGRR
Rhesus macaque	SLYATFLPKWTRPVLPFWKRYLD	PFGSVPFYGVRAACLGRR
Cat	SLYVTFLPKWSRSLLPWKRYLD	PFGSVPFYGVRAAC
Mouse	SVYITFLPKWTRPLLPFWKRYLN	PFGSVPFYGVRAAC
Zebrafish	SMPVVFMPNWTNRYLPFWQRYIG	GFVVRGCVGRRIAELE
Western clawed frog	SVYVTFPLHWTRGILPYWDRIE	PFSSI PFYGVRAACLGK

**Figure 3.** Homologs of the *CYP27A1* gene at the R262 and R474 residues, which are conserved across multiple species.

27-hydroxylase was assessed using bioinformatics tools, including SIFT (<https://sift.bii.a-star.edu.sg/>), Polyphen2 (<http://genetics.bwh.harvard.edu/pph2/>), Mutation Tester (<http://www.mutationtaster.org/>), and CADD (<https://cadd.gs.washington.edu/>), and it was predicted to be highly deleterious (Table). Both amino acids were conserved across the species (Fig. 3). Although the *CYP27A1* genes of the parents were not analyzed, the patient was considered to be compound heterozygous for p.R262C and p.R474W, based on his markedly increased serum cholestanol levels.

The patient was treated with 250 mg/day of CDCA and 10 mg/day of atorvastatin calcium hydrate. However, the dose of CDCA was reduced to 125 mg/day due to drug-induced liver injury. After 21 days of treatment, his serum cholestanol level decreased to 9.1 µg/mL, whereas the clinical manifestations showed no particular improvement.

## Discussion

Spinal form CTX is a rare clinical subgroup of CTX, and

18 patients have been reported to date (4-8, 12, 13). The age of onset ranged from 10 to 40 years, and all patients showed slowly progressive myelopathy, including motor and sensory disturbance of the lower legs (12). Our case had remarkable differences in the onset age, disease progression, and clinical findings, compared to previously reported spinal form CTX patients. Regarding the onset-age, our patient was the oldest among not only spinal form CTX, but also all CTX patients ever reported. Concerning disease progression, Pilo-de-la-Fuente et al. analyzed the duration from onset to diagnosis and EDSS at diagnosis in spinal form CTX patients, which showed that the mean EDSS scores reached 4.4±0.2 (mean±SD) in 18±13 years of disease duration (12). On the other hand, our patient became unable to walk without help (EDSS 7) after a disease duration of only 4 years. The reasons for the rapid progression of symptoms in our patient were unknown; however, excessive alcohol consumption might accelerate spinal cord injury (14). With regard to the clinical findings, our patient solely developed myelopathy without other characteristic manifestations of CTX. Taken

together, our case indicates that CTX should be considered as a differential diagnosis of myelopathy even in elderly-onset and relatively rapidly progressive patients.

A molecular genetic analysis of the *CYP27A1* gene revealed that the patient was heterozygous for the c.784C>T (p.R262C) and c.1420C>T (p.R474W) variant. *CYP27A1* has two functional domains: the adrenodoxin-binding site (residues 351-365) and heme-binding site (residues 435-464). Both sites are critical for sterol 27-hydroxylase activity and most of the missense pathogenic variants are located in these domains (15). The c.1420C>T (p.R474W) is located in the heme-binding site; therefore, this variant is considered to affect the enzyme activity. In contrast, the novel c.784C>T (p.R262C) variant is not related to the functional domains of sterol 27-hydroxylase. However, R262 is a highly conserved amino acid residue (Fig. 3), and c.784C>T (p.R262C) is an infrequent variant in databases on healthy individuals (Table). In addition, *in silico* analyses predicted that this variant considerably alters the structure and function of the enzyme. Based on the clinical findings of our patient and the structural and functional properties of p. R262C, this novel variant is considered to be pathogenic.

CTX is a treatable disease that can follow a good clinical course if an early diagnosis and appropriate treatment are achieved; however, treatment may be less effective once neurological symptoms are fully established (12). Treatment with CDCA reverses metabolic derangement, reduces the serum cholestanol levels, and prevents or even improves neurological dysfunction (4, 12, 16). Competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase also decrease the serum cholestanol levels (17). Indeed, combination therapy of CDCA and HMG-CoA reductase decreased the serum cholestanol level in our patient, although no apparent clinical improvement was observed, probably due to the advanced neurological damage that already existed at the time of presentation.

In summary, we described a 61-year-old pure spinal form CTX patient with a novel variant of the *CYP27A1* gene. CTX should be considered in the differential diagnosis of myelopathy even in elderly-onset and relatively rapidly progressive patients. A greater awareness of CTX and the measurement of the serum cholestanol levels for diagnosis are warranted.

**The authors state that they have no Conflict of Interest (COI).**

#### Financial Support

This study was supported by a grant from the Research Committee for Primary Hyperlipidemia, Research on Measures against Intractable Diseases by the Japanese Ministry of Health, Labour, and Welfare.

#### Acknowledgement

The authors would like to thank Ms. E. Nomura, and Ms. S. Nagasaki for their technical support.

Ken Takasone and Teruya Morizumi contributed equally to this work.

#### References

1. Cali JJ, Hsieh CL, Francke U, Russell DW. Mutations in the bile acid biosynthetic enzyme sterol 27-hydroxylase underlie cerebrotendinous xanthomatosis. *J Biol Chem* **266**: 7779-7783, 1991.
2. Cali JJ, Russell DW. Characterization of human sterol 27-hydroxylase. A mitochondrial cytochrome P-450 that catalyzes multiple oxidation reaction in bile acid biosynthesis. *J Biol Chem* **266**: 7774-7778, 1991.
3. Makishima M, Okamoto AY, Repa JJ, et al. Identification of a nuclear receptor for bile acids. *Science* **284**: 1362-1365, 1999.
4. Sekijima Y, Koyama S, Yoshinaga T, Koinuma M, Inaba Y. Nationwide survey on cerebrotendinous xanthomatosis in Japan. *J Hum Genet* **63**: 271-280, 2018.
5. Nicholls Z, Hobson E, Martindale J, Shaw PJ. Diagnosis of spinal xanthomatosis by next-generation sequencing: identifying a rare, treatable mimic of hereditary spastic paraparesis. *Pract Neurol* **15**: 280-283, 2015.
6. Abe R, Sekijima Y, Kinoshita T, et al. Spinal form cerebrotendinous xanthomatosis patient with long spinal cord lesion. *J Spinal Cord Med* **39**: 726-729, 2016.
7. Saute JA, Giugliani R, Merckens LS, Chiang JP, DeBarber AE, de Souza CF. Look carefully to the heels! A potentially treatable cause of spastic paraplegia. *J Inherit Metab Dis* **38**: 363-364, 2015.
8. Yanagihashi M, Kano O, Terashima T, et al. Late-onset spinal form xanthomatosis without brain lesion: a case report. *BMC Neurol* **16**: 21, 2016.
9. Chen W, Kubota S, Kim KS, et al. Novel homozygous and compound heterozygous mutations of sterol 27-hydroxylase gene (*CYP27*) cause cerebrotendinous xanthomatosis in three Japanese patients from two unrelated families. *J Lipid Res* **38**: 870-879, 1997.
10. Kim KS, Kubota S, Kuriyama M, et al. Identification of new mutations in sterol 27-hydroxylase gene in Japanese patients with cerebrotendinous xanthomatosis (CTX). *J Lipid Res* **35**: 1031-1039, 1994.
11. Nozue T, Higashikata T, Inazu A, et al. Identification of a novel missense mutation in the sterol 27-hydroxylase gene in two Japanese patients with cerebrotendinous xanthomatosis. *Intern Med* **49**: 1127-1131, 2010.
12. Pilo-de-la-Fuente B, Jimenez-Escrig A, Lorenzo JR, et al. Cerebrotendinous xanthomatosis in Spain: clinical, prognostic, and genetic survey. *Eur J Neurol* **18**: 1203-1211, 2011.
13. Verrips A, Nijeholt GJ, Barkhof F, et al. Spinal xanthomatosis: a variant of cerebrotendinous xanthomatosis. *Brain* **122**: 1589-1595, 1999.
14. Sage JI, Van Uitert RL, Lepore FE. Alcoholic myelopathy without substantial liver disease. A syndrome of progressive dorsal and lateral column dysfunction. *Arch Neurol* **41**: 999-1001, 1984.
15. Lee MH, Hazard S, Carpten JD, et al. Fine-mapping, mutation analyses, and structural mapping of cerebrotendinous xanthomatosis in U.S. pedigrees. *J Lipid Res* **42**: 159-169, 2001.
16. Berginer VM, Salen G, Shefer S. Long-term treatment of cerebrotendinous xanthomatosis with chenodeoxycholic acid. *N Engl J Med* **311**: 1649-1652, 1984.
17. Nakamura T, Matsuzawa Y, Takemura K, Kubo M, Miki H, Tarui S. Combined treatment with chenodeoxycholic acid and pravastatin improves plasma cholestanol levels associated with marked regression of tendon xanthomas in cerebrotendinous xanthomatosis. *Metabolism* **40**: 741-746, 1991.

The Internal Medicine is an Open Access journal distributed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License. To view the details of this license, please visit (<https://creativecommons.org/licenses/by-nc-nd/4.0/>).

---

© 2020 The Japanese Society of Internal Medicine  
*Intern Med 59: 2587-2591, 2020*